Restriction Digest Procedure

1. Spin down all enzymes and DNA in the centifuge by pressing the short button until max speed is reached.
2. Put all enzymes, buffers, and DNA on ice.
3. Add 500 ng of DNA to the appropriately labeled tube. Add distilled water to 32 uL.
4. Pipet 5uL of NEB Buffer 2 to each tube.
5. Pipet 1uL of BSA to each tube.
6. In the part A tube, add 1uL of EcoRI and 1uL of SpeI.
7. In the part B tube, add 1 uL of XbaI and 1uL of PstI.
8. In the linearized plasmid tube, add 1uL of EcoRI and 1uL of PstI.
9. Spin all samples briefly to assure that contents have been mixed.
10. Place the samples in the thermal cycler and run the program iGEM3. This program will incubate the tubes at 37ºC for 1 hour and 80ºC for 20 minutes.
    a. Alternatively, place the samples in the 37ºC water bath for 1 hour and an 80ºC water bath for 20 minutes.
11. For the last 20 minutes of the 37ºC incubation, add 1 ul of CIP to the linearized plasmid.
12. Perform gel purification using the QIAGEN gel purification kit and corresponding protocol.

	Part A	Part B	Linearized Plasmid Backbone
DNA	500 ng	500 ng	500 ng (20 uL)
dH2O	to 32 uL	to 32 uL	12 uL
NEB Buffer 2	5 uL	5 uL	5 uL
BSA	1 uL	1 uL	1 uL
Enzyme 1	1 uL EcoRI	1 uL XbaI	1 uL EcoRI
Enzyme 2	1 uL SpeI	1 uL PstI	1 uL PstI